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Electrostatic influence on energetics of electron transfer reactions

(enzyme mechanisms/metalloproteins/protein electrostatics/perfect enzymes)

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ABSTRACT **Electron transfer chains in biological systems must operate efficiently to satisfy metabolic energetic requirements. The component proteins in these chains are expected to exhibit characteristic structural features that facilitate electron transfer to the appropriate donor and acceptor proteins. A survey of soluble one-electron carrier proteins indicates a significant tendency for lower potential proteins to be more negatively charged than higher potential proteins. Consideration of the electrostatic consequences of this pattern of charge asymmetry suggests that the reduction potential difference between the two proteins will be minimized in the precursor complex associated with electron transfer. An equivalent statement is that the change in free energy accompanying electron transfer in the complex will approach zero. This behavior is consistent with theoretical arguments advanced by Albery and Knowles [Albery, W. J. & Knowles, J. R. (1976) *Biochemistry* 15, 5631–5640], which suggest that for the most efficient enzymes, the free energy difference between enzyme-bound species should approach zero. A more general derivation of this prediction is provided. The observed charge asymmetry in electron transfer proteins provides a structural mechanism for satisfying this requirement, thus accelerating the overall rate of electron transfer.**

Proteins in an electron transfer chain must function under the dual constraints of maintaining a rapid flux of electrons, while simultaneously discriminating between a variety of thermodynamically suitable donor and acceptor molecules. The former requirement reflects the central importance of electron transfer processes in the energy metabolism of cells, while the latter feature, by directing electrons along a particular pathway, prevents the system from short-circuiting. In essence, electron transfer proteins are designed to provide low kinetic barriers for transfer to correct acceptors, while maintaining high kinetic barriers against transfer to incorrect acceptors.

The structures of electron-carrier proteins are expected to have evolved so as to facilitate electron transfer to appropriate donor and acceptor proteins (this argument applies to proteins that function only as electron carriers and that have no other activity, such as substrate reduction or ion pumping, coupled to intermolecular electron transfer). For a sequential reaction mechanism, the greatest flux of material occurs for pathways lacking both large kinetic barriers and highly stable intermediates (1). In terms of a free energy diagram, this situation requires that no significant energetic "valleys" or "hills" exist between the initial and final states. As a result, the free energies of all intermediates should decrease smoothly between the reactant and product species. Theoretical arguments advanced by Albery and Knowles (2) suggest that for the most efficient enzymes, the free-energy difference between enzyme-bound species should approach zero. Qualitatively, this principle appears in accord with experimental observations with adenosine triphosphatases (3), kinases (4), and NADH-linked dehydrogenases (5, 6). A more rigorous derivation of this result is presented in the *Appendix*, which indicates that the optimal free-energy profile for electron transfer reactions should have the form illustrated in Fig. 1.

These considerations suggest that optimal electron-transfer rates will occur when the free-energy change for electron transfer in the complex approaches zero. An equivalent statement is that the reduction potential difference between donor and acceptor should also approach zero in the complex. One mechanism for shifting reduction potentials involves changing the electrostatic environment of the redox cofactor (7, 8). To examine a possible role for electrostatic effects of this type in electron transfer, a comparison was made between the molecular charge (as estimated from the protein sequence) and reduction potential for a set of soluble one-electron carrier proteins (Fig. 2). The following discussion is restricted to this class of proteins. A striking result of this survey is the charge polarity of biological electron-carrier proteins. Low potential proteins are, in general, negatively charged, while high potential proteins tend to be more positively charged. A least-squares fit to the data in Fig. 1 gives the following relationship between reduction potential E_0' (in mV) and net charge on a protein (Q)

$$E_0' = 23Q + 205. \quad [1]$$

The correlation coefficient for this relationship is 0.64. Application of Student's t test (19) indicates that the hypothesis that this correlation coefficient is zero can be rejected at the 0.01% level. More convincing is the apparent absence of high potential proteins with molecular charge less than −10

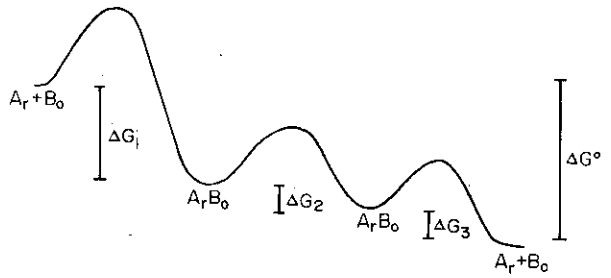

FIG. 1. Free-energy profile for electron transfer between reduced and oxidized forms of proteins A and B (designated A_r, A_o, B_r, and B_o, respectively). Electron transfer takes place between the central A_rB_o and A_oB_r complexes. For optimal rates of electron transfer, the equilibrium constants corresponding to ΔG_1, ΔG_2, and ΔG_3 are given by $K_1 = [k_dK_e/2k_{et}(S)]^{1/2}$; $K_2 = 1$; and $K_3 = K_e/K_1$, where k_d, K_e, k_{et}, and (S) are the bimolecular diffusion-controlled rate constant, overall equilibrium constant, first-order rate constant for electron transfer, and physiological concentration of electron carriers, respectively. For a "typical" electron transfer reaction, $k_{et} = 10^2\ sec^{-1}$, $k_d = 10^9\ M^{-1}\cdot sec^{-1}$, $(S) = 10^{-5}$ M, and $K_e = 50$. With these parameters, $K_1 = 5 \times 10^6\ M^{-1}$ and $K_3 = 10^{-5}$ M. If the physiological concentration of carriers is selected as standard state (2), then ΔG_1, ΔG_2, $\Delta G_3 = -2.3$, 0, 0 kcal/mol, respectively.

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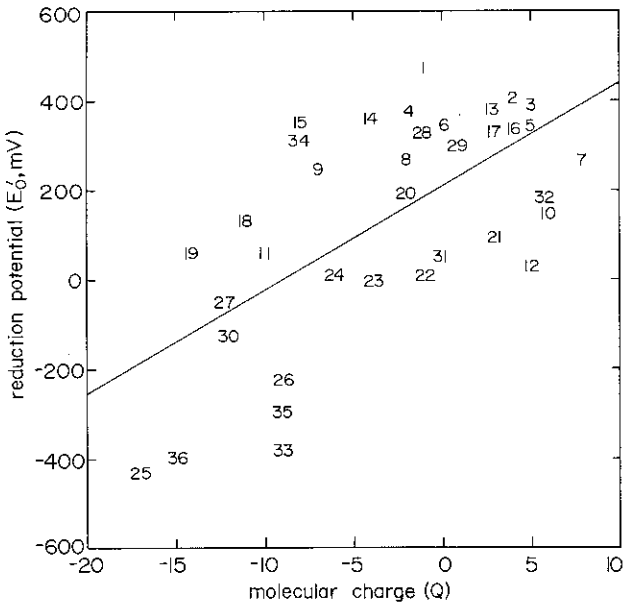

FIG. 2. Relationship between reduction potential (E_0') and molecular charge (Q) for a series of soluble one-electron carrier proteins. Data points 1–31 represent cytochromes, high potential iron proteins, ferredoxins, azurins, flavodoxin, and myoglobin listed in tables 1 and 2 of ref. 9. Remaining proteins are as follows: 32, *Rhus* stellacyanin (10, 11); 33, bovine adrenodoxin (12, 13); 34, spinach plastocyanin (14); 35, *Clostridium pasteurianum* nitrogenase iron protein (15, 16); 36, *Azotobacter vinelandii* nitrogenase iron protein (17, 18). The least-squares line through these data points is shown. The influence of asymmetric charge distributions has not been taken into consideration.

and low potential proteins with molecular charge greater than −5. As a consequence, biological electron-transfer chains exhibit a significant tendency to transfer electrons from more acidic lower potential proteins toward more basic higher potential proteins.

The observation that electron transfer tends to occur between acidic donor and basic acceptor proteins is consistent with the free-energy considerations discussed above. In the electron transfer complex, negative groups on the donor will tend to "neutralize" positive groups on the acceptor. Neutralization of negatively charged groups will lower the stability of the oxidized (more positively charged) form of the donor redox group, thus raising the reduction potential of the donor in the complex. Likewise, neutralization of positive groups will increase the stability of the oxidized form of the acceptor redox group, thus lowering the reduction potential of the acceptor in the complex. Consequently, complex formation will decrease the difference between the donor and acceptor reduction potential, thus serving to accelerate the overall rate of electron transfer.

For electron transfer reactions, the concept of equal reduction potential applies not only to bound species in the collision complex, but also to activated complexes surrounding the transition state. A central feature in the electron-transfer reaction pathway is the formation of an activated complex between donor and acceptor molecules in which electron exchange actually occurs (20). The time scale for the actual electron transfer (10^{-15} sec) is much more rapid than the time scale of nuclear motion (10^{-13} sec). As a consequence, electron transfer is subject to restrictions imposed by the Franck–Condon principle, which requires that nuclear positions remain fixed during electron exchange. Libby (21) and Marcus (22) demonstrated that this restriction implies that the free energy of the system must remain fixed during the electron transfer process. Since the reactant-activated complex and the product-activated complex both have the same free energy, the reduction potentials for donor and acceptor species in the activated complex must also be equal.

The magnitude of the reduction potential change upon complex formation may be estimated from the following considerations. Complex formation between electron transfer proteins often appears to be stabilized by salt bridge formation between the carriers in the region of the redox cofactors (23). To a first approximation, salt bridge formation results in neutralization of two charges (neglecting the resultant dipole that is generated). The effect of charge neutralization on reduction potential can be estimated from chemical modification studies. Neutralization of single lysine residues near the heme crevice of cytochrome *c* reduces the reduction potential by ≈15 mV (24). When ionic strength effects are considered, the change is closer to 25 mV (25). Methyl esterification of both heme propionate groups in cytochrome b_5 (resulting in the loss of two negative charges) leads to an increase in reduction potential of 60 mV (26). Consequently, the absolute magnitude for the shift in reduction potential for proteins in a complex might be expected to be 15–30 mV times the number of salt bridges formed with the partner molecule. This estimate is also in agreement with the value of 23 mV obtained from the slope term dE_0'/dQ of Eq. **1** for the variation in reduction potential with molecular charge for redox proteins.

An order-of-magnitude estimate for the dependence of reduction potential on molecular charge can also be obtained by a simple theoretical calculation, using a naive electrostatic model for a protein. If a protein is described as a sphere of radius R, with a net charge Q smeared uniformly about the surface, and immersed in a medium of dielectric constant ε, the total electrostatic free energy G_{el} of the protein is given by (27)

$$G_{el} = \frac{e^2Q^2}{2\varepsilon R}, \qquad [2]$$

where e is the protonic charge. The difference in electrostatic free energy for charging the reduced and oxidized forms of a redox protein is given by (28)

$$\Delta G_{el} = \frac{e^2}{2\varepsilon R}\,(Q_{red}^2 - Q_{ox}^2), \qquad [3]$$

where Q_{red} and Q_{ox} are the net charge of the reduced and oxidized forms of the enzyme, respectively. When $Q_{red} = Q_{ox} - 1$ (neglecting possible oxidation state-linked ion binding, etc.), Eq. **3** reduces to

$$\Delta G_{el} = \frac{e^2}{2\varepsilon R}\,(1 - 2Q_{ox}). \qquad [4]$$

Converting to reduction potential gives (for a one-electron carrier protein)

$$E_0' = \frac{-e^2}{2F\varepsilon R}\,(1 - 2Q_{ox}), \qquad [5]$$

where F is the Faraday constant.

The variation in reduction potential with charge is given by the term dE_0'/dQ_{ox}

$$\frac{dE_0'}{dQ_{ox}} = \frac{e^2}{F\varepsilon R}. \qquad [6]$$

With $\varepsilon = 80$ (water) and $R = 15 \times 10^{-8}$ cm, dE_0'/dQ is calculated to be 12 mV. The agreement with the experimental esti-

mates is quite satisfactory, given the simplistic nature of the protein model.

Taken together, the experimental and theoretical estimates suggest that neutralization of only a few charged groups in the activated complex can lead to shifts in reduction potentials on the order of 100 mV. If both proteins experience this shift, the overall difference in reduction potential will change by 200 mV. Thus, the electrostatic effects outlined above are of sufficient magnitude to bring about the required shifts in reduction potential during the electron transfer reaction.

The charge asymmetry observed between donor and acceptor proteins in biological electron transfer systems provides an intermolecular contribution toward accelerating the electron exchange rate. Given the structural complexity of proteins, however, it should not be assumed that the catalytic efficiency of electron-transfer proteins is due entirely to these electrostatic influences. Experimental (29) and computational (30) studies indicate that the intramolecular reorganizational enthalpy associated with structural changes during electron transfer is small. Both intra- and intermolecular contributions toward reducing the activation barrier for electron transfer are critical, and it is to be anticipated that one effect or the other may dominate for a particular pair of donor and acceptor proteins.

A prominent role for electrostatic interactions in stabilizing electron transfer complexes has been previously documented (see, for example, refs. 23, 24, and 31–33). The formation of a precursor complex stabilized by complementary charge and dipole interactions can accelerate the reaction rate by helping to steer proteins toward each other and stabilizing the resulting complex in an orientation favoring electron transfer. Although this aspect of electrostatic influence on electron-transfer rates requires that the donor and acceptor molecules have complementary charge distributions, there is no apparent requirement for donors to be more negatively charged than acceptors. Electrostatic stabilization of the precursor complex is an important consequence of the charge asymmetry observed between donor and acceptor proteins, but this effect is distinct from the proposed electrostatic contribution toward optimizing the free-energy profile of electron transfer.

APPENDIX

"Perfect" Enzymes: The Equilibrium Constant Between Bound Species Is Unity. As discussed by Albery and Knowles (2), an enzyme approaches catalytic perfection when the rate of the reaction is controlled by diffusive steps. The achievement of perfection is envisioned as proceeding through three major stages designated "uniform binding," "differential binding," and "catalysis of elementary steps." An interesting prediction by Albery and Knowles was that for enzymes optimized with respect to the first two stages, the equilibrium constant would be unity between the kinetically significant bound species surrounding the kinetically significant transition state. This point was challenged by Chin (34), however, who noted that the original derivation contained several errors. The purpose of this note is to demonstrate that the equilibrium constant for bound species is unity for enzymes optimized with respect to all three stages.

Attempts to maximize the overall reaction velocity, k, with respect to the elementary rate constants for the enzyme catalyzed reaction

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} EP \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} E + P \qquad \textbf{[A1]}$$

lead to physically meaningless results, such as negative or imaginary rate constants. Two restrictions on rate constants have been introduced in the kinetic analyses of perfect enzymes (2, 34): (*i*) biomolecular steps represented by rate constants k_1 and k_{-3} are assumed to occur with the diffusion-limited rate constant k_d; and (*ii*) a linear free-energy relationship holds for the elementary catalytic step $k_2 = CK_2^\beta$, where C is a constant, β is the Bronsted coefficient, and $K_2 = k_2/k_{-2}$.

With these substitutions, $1/k$ for the irreversible reaction case may now be written as a function of the variables K_1, K_2, and β [where the equilibrium constant K_e, (S), k_d, and C are constants]

$$\frac{1}{k} = \frac{1}{(S)}\left[\frac{1}{k_d} + \frac{1}{CK_1K_2^\beta} + \frac{1}{k_dK_e}\right] + \frac{1}{CK_2^\beta} + \frac{K_1}{k_dK_e}(K_2 + 1). \qquad \textbf{[A2]}$$

The value of (S) is taken as the physiological concentration of substrate. The problem of defining perfection then reduces to finding the values of K_1, K_2, and β for which $1/k$ is a minimum. This occurs when the total derivative of $1/k$ vanishes. Calculation of the appropriate derivatives yields

$$\frac{\partial\frac{1}{k}}{\partial K_1} = -\frac{1}{CK_1^2K_2^\beta(S)} + \frac{K_2 + 1}{k_dK_e} = 0 \qquad \textbf{[A3a]}$$

$$\frac{\partial\frac{1}{k}}{\partial K_2} = -\frac{\beta}{CK_2^{\beta+1}}\left(\frac{1}{K_1(S)} + 1\right) + \frac{K_1}{k_dK_e} = 0 \qquad \textbf{[A3b]}$$

$$\frac{\partial\frac{1}{k}}{\partial \beta} = -\left(\frac{1}{K_1(S)} + 1\right)\frac{\ln K_2}{CK_2^\beta} = 0. \qquad \textbf{[A3c]}$$

For finite K_2, Eq. **A3c** vanishes only when $\ln K_2 = 0$, or $K_2 = 1$. Thus, for an enzyme optimized with respect to β (catalysis of elementary step), the equilibrium constant, K_2, between enzyme-bound species is unity. Under these conditions, C is equivalent to the catalytic rate constant k_2.

With the substitution $K_2 = 1$, Eqs. **A3a** and **A3b** may be solved for β and K_1, giving

$$K_1 = \left[\frac{k_dK_e}{2C(S)}\right]^{1/2} \qquad \textbf{[A4a]}$$

$$\beta = \frac{1}{2}\left[\frac{1}{1 + \left(\frac{k_dK_e(S)}{2C}\right)^{1/2}}\right] \qquad \textbf{[A4b]}$$

These expressions describe the dependence of the reaction profile on various fundamental (k_d and K_e) and environmental (S) parameters. K_1, the association constant for enzyme and substrate, will be large (tight binding) when K_e is large (large driving force for the reaction), or when (S) is small (low concentration of substrate). As (S) increases, K_1 decreases, corresponding of less tight binding of enzyme and substrate. Under these conditions, complex formation is driven by the high concentration of (S). K_3 (the dissociation constant for enzyme and product) will vary inversely with K_1, since $K_3 = K_e/K_1$ when $K_2 = 1$. From Eq. **A4b**, the value of β is seen to vary between 0 and 1/2. According to an interpretation of the Hammond postulate (35–37), the value of β indicates how closely the transition state resembles the reactants ($\beta = 0$), products ($\beta = 1$), or an intermediate structure ($0 < \beta < 1$). For large values of the overall driving force K_e, β tends to zero, so the transition state resembles the substrate. Although β corresponds to the elementary step inter-

converting enzyme bound species of equal free energy, it is actually sensitive to the free-energy difference between the free species.

For the case of a reversible reaction scheme, an analogous derivation yields

$$K_1 = \left[\frac{k_d K_e}{2C(S)(K_e + 1)}\right]^{1/2} \quad \textbf{[A5a]}$$

$$K_2 = 1 \quad \textbf{[A5b]}$$

$$\beta = \frac{1}{2}. \quad \textbf{[A5c]}$$

Subject to the restrictions imposed by the condition $k_2 = CK_2^{\beta}$, the conclusion that the equilibrium constant between bound species is unity is general for catalytically optimal enzymes operating under both reversible and irreversible conditions.

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